Acetylcholinesterase Enzyme Activity in Fish Sampled after the 1985 Newfoundland Forest Spray Program for Eastern Hemlock Looper using Fenitrothion

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ACETYLCHOLINESTERASE ENZYME ACTIVITY IN FISH SAMPLED AFTER THE 1985 NEWFOUNDLAND FOREST SPRAY PROGRAM FOR EASTERN HEMLOCK LOOPER USING FENITROTHION

by

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ABSTRACT

Fancey, L. L., J. F. Payne, M. A. Barnes, and R. N. McCubbin. 1987. Acetylcholinesterase enzyme activity in fish sampled after the 1985 Newfoundland forest spray program for eastern hemlock looper using fenitrothion. Can. Tech. Rep. Fish. Aquat. Sci. 1579: iv + 10 p.

During the summer of 1985 two sites in insular Newfoundland were sprayed with fenitrothion by the Government of Newfoundland to reduce the hemlock looper (Lambdina fiscellaria fiscellaria (Guen)) population. Two spray applications using 210 g a.i.·ha^{-1} were applied with 400 m buffer zones being left around streams. Atlantic salmon (Salmo salar) parr and juvenile brook trout (Salvelinus fontinalis) were sampled at various times pre- and post-spray and assays for levels of brain acetylcholinesterase activity were carried out. No differences in enzyme activity were detected between control and spray sites. Results support the hypothesis that the present operational conditions of low dose and adequate buffer zones afford a reasonable level of protection for fish from any direct toxic effects of the pesticide fenitrothion.

RÉSUMÉ

Fancey, L. L., J. F. Payne, M. A. Barnes, and R. N. McCubbin. 1987. Acetylcholinesterase monitoring in fish sampled after the 1985 Newfoundland forest spray program for eastern hemlock looper using fenitrothion. Can. Tech. Rep. Fish. Aquat. Sci. 1579: iv + 10 p.

Au cours de l'été 1985, des employés du gouvernement de Terre-Neuve ont effectué des pulvérisations de fénitrothion à deux endroits afin d'y contrôler la population d'arpenteuse de la pruche (Lambdina fiscellaria fiscellaria (Guen)). Ces sites ont été pulvérisés deux fois à un taux de 210 g a.i.·ha^{-1} sauf pour une zone tampon de 400 m en bordure des cours d'eau. Les tacons de saumon de l'Atlantique (Salmo salar) et les juvéniles de truite arc-en-ciel (Salvelinus fontinalis) échantillonnés avant et après les pulvérisations ont fait l'objet d'une bio-analyse de l'activité de l'acétylcholinestérase céphalique. Aucune différence n'a été observée entre les endroits pulvérisés et les sites témoins pour ce qui est de l'activité enzymatique. Les résultats étayent l'hypothèse que le régime actuel de pulvérisation à faible dose assorti de la création de zones tampon adéquates offre un niveau raisonnable de protection contre toute incidence directe toxique du fénitrothion dans le cas des poissons.

INTRODUCTION

In 1985 an aerial spray program against the eastern hemlock looper (Lambdina fiscellaria fiscellaria (Guen)) was conducted with the insecticide fenitrothion by the Government of Newfoundland and Labrador Department of Forest Resources and Lands. The spray program included areas which were close to rivers and streams that contained fish potentially sensitive to effects of the pesticide.

Brain acetylcholinesterase (ACHE) activity has been shown to be a sensitive indicator of pesticide poisoning (Coppage and Braidech 1976). Assays to determine ACHE activity were performed on samples of fish taken from the area involved in the aerial spray program. Reduction of brain ACHE in fish collected in the spray zone would indicate fenitrothion (or degradation product) contamination of the stream area at concentration levels sufficient for systemic poisoning.

METHODS AND MATERIALS

1. SPRAY PROGRAM

An aerial spray program using fenitrothion was applied at 28 sites in insular Newfoundland during the summer of 1985. Areas were sprayed twice at an application rate of 210 g. a.i. ha^{-1} and a 400-meter no-spray buffer zone was implemented to protect aquatic habits. The composition of the operational spray mix was 11% fenitrothion (O,O-dimethyl O-(3 methyl-4-nitrophenyl phosphorothioate), 40% Cyclosol 63 and 49% insect diluent 585 or common stove oil.

2. FISH SAMPLING

Atlantic salmon parr (Salmo salar) and juvenile brook trout (Salvelinus fontinalis) 4 to 16 cm were collected by electrofishing before and after spraying at two sites, one at Frenchman's Brook in Central Newfoundland and another at Colinet River on the Avalon Peninsula (Fig. 1). Control fish samples were similarly collected from untreated areas at a tributary of the Exploits River in Central Newfoundland and at North Harbor River on the Avalon Peninsula near the Colinet River (Fig. 1). Frenchman's Brook fish were taken from within the spray block and Colinet River samples were collected immediately downstream of the southern extent of the spray block.

Fish samples were taken 24 h after spraying at the Frenchman's Brook and Exploits Tributary sites and at both 48 h and 96 h after spraying at the Colinet River. Post-spray samples at the North Harbor River were taken 48 h after spraying.

3. BRAIN HOMOGENATE PREPARATION

Fish samples were frozen on dry ice while being transported to the laboratory and were then held at -80°C until analyzed.

The brain of each fish was dissected while frozen and five brains from fish of similar length were pooled. The pooled sample was then weighed and homogenized in 10 volumes of Trizma buffer per gram of brain. The homogenate was centrifuged at 10,000 g for 10 min and the supernatant fraction was assayed for ACHE activity using acetylthiocholine iodide as substrate and 5, 5-Dithiobis-(2-nitrobenzoic acid, DTNB) as chromogen reagent (Ellman et al. 1961; Hill and Fleming 1982; Ernst and Julien 1984).

4. REAGENT PREPARATION

Buffer: The buffer consisted of 4.4 g Trizma HCl and 2.65 g Trizma base made up to 10 ml with deionized water and stored at room temperature. The pH of the buffer was 7.6.

Substrate: Acetylthiocholine iodide 451.1 mg was made up to 10 ml with deionized water. This solution was made up daily.

Chromogen Reagent: The chromogen reagent consisted of 9.9 ml DTNB, 661.0 mg Trizma HCl and 97.0 mg of Trizma base made up to 100 ml with deionized water. This solution is stable refrigerated in the dark for two months.

Trizma HCl, Trizma base, DTNB and acetylthiocholine iodide were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. 63178.

5. SPECTROPHOTOMETRY

A Perkin-Elmer recording scanning spectrophotometer, Model 571, was used to measure product formation. Cuvettes of 1 cm optical patch length were used and measurements were made at 405 nm wavelength. To each sample cuvette 2.9 ml of chromogen reagent, 100 μl of acetylthiocholine solution and 20 μl of homogenate solution was added. No homogenate solution was added to the reference cuvette. Change in absorbance was measured at room temperature (25°C) over a one minute period while the reaction is in progress.

During the reaction ACHE hydrolyzes acetylthiocholine iodide into thiocholine and acetate. Thiocholine reacts with dithiodinitrobenzoic acid to form thionitrobenzoic acid which is yellow and is measured at 405 nm on a spectrophotometer. The rate at which color is produced indicates the level of ACHE activity.

Enzyme activity is calculated by multiplying the change in absorbance over one minute by 130 to provide micromoles of substrate per minute per gram of brain (wet weight) (Hill and Fleming 1982).

6. STATISTICAL ANALYSES OF DATA

No statistical analyses were performed on pooled samples of less than 5 fish. One pool of prespray brook trout from the tributary of the Exploits River contained 7 fish.

All brook trout data were analyzed using a nested analysis of variance (ANOVA) procedure. Both the Avalon Peninsula and central Newfoundland locations had spray and control sites nested within and in turn each spray and control site had a pre- and post-spray sample nested within.

Atlantic salmon data were only available for the central Newfoundland locations so no comparisons were possible with Avalon Peninsula sites. Only pre- and post-spray comparisons were possible for central Newfoundland salmon.

Also in the brook trout data for each of the Avalon Peninsula and central areas the pre-spray sample in the spray block and the pre- and post-spray samples in the control area may all be considered as "nontreatment" samples. Therefore, the two spray block ACHE activity means are collectively compared to the 6 "nontreatment" sample means using a multiple means comparison.

RESULTS

The results of the nested ANOVA procedure on brook trout show that no significant difference exists in ACHE activity between the central and Avalon Peninsula areas of Newfoundland (α=0.05) at 24 h and 48 h post-spray respectively. Also, no significant differences in activity exist in treatment, i.e. between the central and Avalon Peninsula areas (Table 1).

The results of the multiple mean comparison on the brook trout data confirm that no significant differences exist. Also, no significant differences in ACHE activity exist in treatment (between control and spray fish) for central Newfoundland salmon.

DISCUSSION

Fenitrothion is an organophosphate insecticide used to control insect pests in the forest industry. It is a nerve poison which acts by blocking synaptic transmission in cholinergic parts of the nervous system. The insecticide binds to the active site of the enzyme ACHE and prevents the breakdown of the neurotransmitter acetylcholine causing its accumulation (Verma et al. 1979). Fenitrothion, like other organophosphates and carbamate compounds, is metabolized by most vertebrates and does not appear to accumulate (Hill and Fleming 1982). Metabolites in fish include 3-methyl-4-nitrophenol, its glucuronide, desmethyl fenitrothion, desmethylfenitrooxon and fenitrooxon (Takimoto and Miyamoto 1976).

Fenitrothion is readily degraded in fish and it is possible that photodecomposition may increase the rate of decomposition in water. Malis and Muir (1980) in their experiments in test ponds found that 50% of the

disappearance of fenitrothion may be attributed to photolysis. Morrison and Wells (1981) found, however, that fenitrothion may be quite stable in acid waters and that photodecomposition is reduced in peat laden waters.

During an operational spray for forest pests some spray drift may bring the pesticide in contact with waters supporting fish populations. The effect of such introduction of organophosphates into a biological system may be examined in several ways. The concentration of chemical in the water may be measured and monitored before and after a spray program. For fenitrothion the levels which define lethal thresholds are not normally reached in waterways around spray areas. For instance in this study the levels of pesticide in Frenchman's Brook declined from .004 mg l^{-1} 10 min post spray to 0.003 mg l^{-1} 96 h post spray during the first application and from 0.0212 mg l^{-1} 30 min post spray to 0.002 mg l^{-1} 48 h post spray during the second application (information provided by Environmental Protection Service, St. John's). Even if concentrations of fenitrothion in water are determined to be well below threshold levels for toxicity, it can be argued that actual levels may be underestimated due to photodecomposition processes or that various types of environmentally or biologically produced degradation products may have real importance in toxicity (in addition to any effects of the parent compound per se). Sensitive biological indicators can be used to assess the "complement" of toxicities which may arise in such circumstances. Since the principal mechanism of toxicity of organophosphate pesticides is inhibition of the enzyme acetylcholinesterase in brain and nerve tissues, changes in the catalytic activity of this particular enzyme can be used to assess potential toxicity.

A number of workers have investigated the effects of pesticides on ACHE enzyme levels in fish. Weiss and Gakstatter (1964) suggested that acetylcholinesterase enzyme monitoring be used to indicate organophosphate pesticides in fish in natural waters. Reduction in enzyme activity would indicate presence of a neurotransmitter blocking agent. It is possible to detect previous pollution with this technique because ACHE levels after exposure to organophosphate pesticides may take a month or more to return to normal (Weiss 1961). Studies carried out by Hatfield (1969) and Zitko et al. (1970) on the effects of aerial spraying of fenitrothion and similar pesticides on salmon and brook trout showed little if any detrimental effects of the pesticide on fish brain acetylcholinesterase or fish mortality in general. Also Coady (1978) found no evidence for direct toxicological effect on fish in his study with fenitrothion and Aminocarb. Coppage and Braidech (1976) suggested that brain ACHE measurement in fish is a sensitive and reliable indicator of organophosphate and carbamate pesticides. Fish treated with sublethal amounts of Malathion could lose about 30% of their cholinesterase activity with little loss of ability to maintain a fixed position in a water current (Post and Leasure 1974). Greater reductions in cholinesterase activity were associated with reduced stamina. Hill and Fleming (1982), in their work with the measurement of brain ACHE activity in birds, suggested that 20% depression of activity indicates exposure to anti-ACHE chemicals and studies with fish tend to support the 20% criterion (Weiss 1958; Williams and Sova 1966; Holland et al. 1967; Nicholson 1967;). Lockhart et al. (1985) suggested that the interpretation of individual sublethal cholinesterase activity monitoring should be calibrated in terms of population responses. He sampled a population of fish exposed to Malathion for survival and growth data and

related these parameters to effects on cholinesterase activity. He concluded that a reduction in cholinesterase activity to about 25% of pre-exposure levels was the approximate threshold for population effects.

In this study a reduction to 25% of pre-exposure levels of ACHE activity was taken as the point at which we might expect an individual sublethal response such as enzyme activity reduction to be a significant indicator of effects at the population level. Our results indicated no significant differences in ACHE activities in the brains of salmon parr and brook trout sampled before and after operational spraying of fenitrothion. Acetylcholinesterase enzyme activities of fish in control areas were similar to those in the corresponding spray treatment areas. Similarly, Morrison and Wells (1981) found no evidence that the resident fish population in a spray area stream was affected as shown by the relatively little change in brain acetylcholine activity. In their study, fenitrothion was applied at a rate of 300 $g \cdot ha^{-1}$ from fixed wing aircraft flying in 50-meter swathers. This application gave a concentration of fenitrothion in a forest stream to a maximum of 0.018 mg l^{-1} within 1 h after spraying but fell to 0.0005 mg l^{-1} after 24 h.

Wildish et al. (1971) estimated that the concentration of fenitrothion in an average stream under operational spraying is in the order of 0.045 $mg \cdot \ell^{-1}$, while the lethal threshold is 2.0 $mg \cdot \ell^{-1}$. Yet it is possible that some sublethal neurological effect at the concentrations provided by operational spraying of streams could translate into effects at the population level. Sublethal effects could include inhibition of learning ability in Atlantic salmon parr or increased predation by brook trout on affected parr (Hatfield and Johansen, 1972). The effect on fish may be indirect such as the reduction of food ration of available insects after an operational spray program but it has been noted that it is unlikely that insects consumed after being killed by operational spray doses of fenitrothion would cause lethal or sublethal effects (Wildish and Lister 1973). Direct effects of fenithrothion poisoning may include lethargy, loss of equilibrium and hyperactivity (Benke and Murphy 1974). Organophosphates may also impair a fish's ability to sustain physical activity in search of food by upsetting their body stability in fast flowing streams and by impairing ability to escape enemies (Verma et al. 1979). Our results indicate that none of these putative neurological effects likely occurred to any extent in fish as a result of the 1985 spray program in Newfoundland.

SUMMARY

Fenitrothion is absorbed into fish through gill filaments and perhaps through skin directly from water (Lockhart et al. 1973; Takimoto and Miyamoto 1976). Anti cholinergic responses may be evidenced and reduction in cholinesterase activity may follow. In addition, various environmental or biological degradation products of fenitrothion may enter fish either directly or indirectly through consumption of contaminated prey. In this study, if toxicologically significant amounts of the parent pesticide compound or its metabolites were present in fish at the time of sampling a corresponding reduction in acetylcholinesterase activity would be expected. From the results

presented here, such was not the case for streams sampled in the spray zone for hemlock looper in 1985. Overall, our results indicate that the present operational conditions of low dose and adequate buffer zones offer a reasonable level of protection for fish from any direct toxic effects of the pesticide Fenitrothion. However, we recommend (as for any pesticide, chemical or biological) that the situation be continuously assessed and monitored.

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Table 1. Brain acetylcholinesterase activity in fish from spray and control areas, before and after spraying (u moles/min/g wet weight).

	Avalon Peninsula					Central Newfoundland							
	Control Area North Harbour River		Spray area Colinet River			Control area Exploits Tributary				Spray area Frenchman's Brook			
				96 h after 1st spray	48 h after 2nd spray	Pre-spray		Post spray		Pre-spray		Post-spray	
	Pre-spray Brook trout	Post-spray Brook trout	Pre-spray Brook trout	Brook trout	Brook trout	Brook trout	Salmon	Brook trout	Salmon	Brook trout	Salmon	Brook trout	Salmon
1	8.97	12.09	6.83	9.75	7.80	12.87	9.75	10.92	10.53	12.87	12.29	12.09	10.14
2	10.92	11.70	10.14	12.09	8.19	12.09	15.99	7.61	10.14	13.65	-	9.75	9.36
3	10.53	11.70	11.31	10.92	10.14		21.45	10.14	9.56	12.48	10.53	13.26	10.92
4	16.77	12.48	10.14	10.53	9.17		14.43		11.31	13.65	10.92	13.65	8.39
5	12.48		11.31	11.51	7.6		10.92		11.90	9.75		13.65	
6	11.70		10.53	10.14	11.51		7.41						
7							8.78						
8							10.53						
9							10.92						
10													
	$\bar{x}$=11.895 SD=2.67	$\bar{x}$=11.99 SD=0.37	$\bar{x}$=10.04 SD=0.87	$\bar{x}$=10.82 SD=0.87	$\bar{x}$=9.06 SD=1.53	$\bar{x}$=12.48 SD=0.39	$\bar{x}$=11.92 SD=4.23	$\bar{x}$=9.56 SD=1.73	$\bar{x}$=10.69 SD=0.93	$\bar{x}$=12.48 SD=1.61	$\bar{x}$=10.68 SD=1.37	$\bar{x}$=12.48 SD=1.66	$\bar{x}$=9.70 SD=1.08

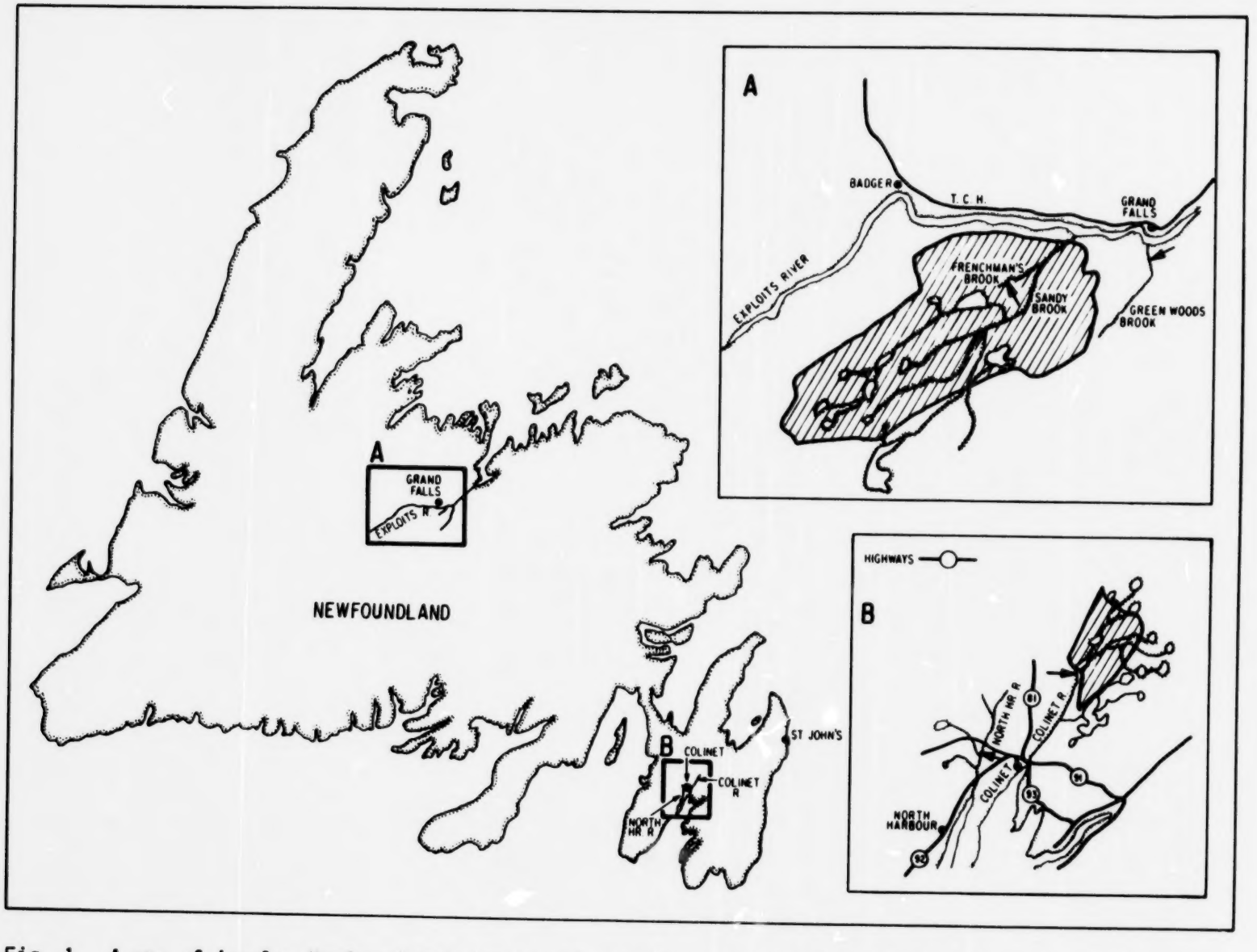

Fig. 1. A map of insular Newfoundland with inserts showing exact sampling sites as denoted by the arrows. Cross-hatched areas within the inserts A and B denote the spray blocks.